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Assessment of persistent brominated and chlorinated organic contaminants in the European eel (*Anguilla anguilla*) in Flanders, Belgium: Levels, profiles and health risk

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HIGHLIGHTS

- PCBs are still the major contaminants in most of the 60 pooled eels from Flanders.
- PBDE and HBCD intakes through eel consumption were <RfD for the average population.
- At 16/60 sites, eels exceeded 300 ng/g ww the new EU consumption threshold for PCBs.
- There is proof of on-going exposure of Flemish eels to PBDEs, HBCDs, PCBs and DDTs.

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ABSTRACT

Pooled yellow European eel (*Anguilla anguilla* (L.)) samples, consisting of 3–10 eels, collected between 2000 and 2009 from 60 locations in Flanders (Belgium) were investigated for persistent contaminants, such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane and its metabolites (DDTs). The current study expands the knowledge regarding these contaminant concentrations, their patterns and distribution profiles in aquatic ecosystems. PBDEs, HBCDs, PCBs, and DDTs were detected in all eel samples and some samples had high concentrations (up to 1400, 9500, 41,600 and 7000 ng/g lw, respectively). PCB levels accounted for the majority of the contamination in most samples. The high variability in PBDE, HBCD, PCB and DDT concentrations reported here is likely due to the variety in sampling locations demonstrating variable local pollution pressures, from highly industrialised areas to small rural creeks. Among PBDEs, BDE-47 (57% contribution to the sum PBDEs), −100 (19%) and −99 (15%) were the predominant congeners, similar to the composition reported in the literature in eel samples. For HBCDs, α-HBCD (74%) was predominant followed by γ-(22%) and β-HBCD (4%) isomers in almost all eel samples. CB-153 (19%) was the most dominant PCB congener, closely followed by CB-138 (11%), CB-180 (9%), CB-187 (8%) and CB-149 (7%). The contribution to the total human exposure through local wild eel consumption was also highly variable. Intake of PBDEs and HBCDs, through consumption of wild eel, was below the RfD values for the average population (consuming on average 2.9 g eel/day). At 16 out 60 sites, eels exceeded largely the new EU consumption threshold for PCBs (300 ng/g ww for the sum of 6 indicator PCBs). The current data shows an on-going exposure of Flemish eels to PBDEs, HBCDs, PCBs and DDTs through indirect release from contaminated sediments or direct releases from various industries.

1. Introduction

The steep decline in populations of eels (*Anguilla* spp.) endangers the immediate future of these legendary fish. In recent decades, juvenile abundance has declined dramatically by 95% for the European eel (*Anguilla anguilla*) and by 80% for the Japanese eel (*Anguilla japonica*). Recruitment of American eel (*Anguilla rostrata*) to Lake Ontario, near the species' northern limit, has virtually ceased (Dekker et al., 2003). Several suggestions have been made on the reasons of this decline: extensive fisheries on all life stages, climate change, pollution, endocrine disruption, insufficient energy for migration, oceanic changes, habitat loss, migration barriers, or diseases (ICES, 2002; Belpaire et al., 2009).

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Due to its complex life cycle, very little is yet known about the eel's reproduction. It has been suggested that reproductive failure, if taking place, is due to fewer eels reaching the Sargasso Sea as a result of low fat content and related energy reserves (Belpaire et al., 2009) or due to effects of chemicals on the reproduction itself (Palstra et al., 2006). Two large and independent data sets from Belgium and the Netherlands showed a one-third decrease in fat content, on average, in yellow eels over the past 15 years, and this decrease could have been caused by bioaccumulation of toxic substances during the growth stage of the eel in continental waters (Belpaire et al., 2009). However, a direct relationship between the reported effects and a population level response has not yet been established.

Among fishes, European eels are unique in their reproductive behaviour and migrate once a year uncommon large distances to reach their spawning sites. As silver eels they leave the European and North African coasts in the fall and are supposed to reach the Sargasso Sea after about 6 months (Tesch, 2003). Prior to maturation and migration back to their spawning grounds, eels undergo a silvering process accompanied by drastic physiological changes, inclusive degeneration of the alimentary track (Durif et al., 2005). Therefore, at the start of the migration, silver eels stop feeding and the gonads start developing. Eels have much fat as energy stores, which have been reported to be sufficient to reach their spawning grounds (van Ginneken and van den Thillart, 2000). However, for their long-distance migration to the Sargasso Sea, the energy reserves may easily become critical particularly since the fat percentage varies largely (Svedäng and Wickstrom, 1997). From the spawning area in the Sargasso Sea, the eel larvae reach continental waters where they grow up in fresh water and coastal habitats during their sedentary yellow eel phase.

Humans are exposed to a cocktail of environmental chemicals including the legacy and emerging persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), and brominated flame retardants (BFRs), e.g. polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs). Elevated PBDE levels measured in various environmental and biological samples led to the ban of penta- and octa-BDE technical mixtures, due to growing apprehension and, the two have been added to the list of POPs established by the United Nations Stockholm Convention since May, 2009 (Bromine Science and Environmental Forum (BSEF), 2010). However, deca-BDE technical product is still used mainly in plastic housing for electrical and electronic equipment (BSEF, 2007). HBCDs are also widely used in a variety of industrial and household appliances, making this compound the second most used BFR in Europe and the third most used BFR worldwide due to their possible application as an alternative for PBDEs (VECAP, 2013). HBCDs have become a cause for concern due to their persistence, bioaccumulative nature, toxicity and their scale of use (Covaci et al., 2006). Consequently, HBCD has been included as a new POP in 2013 (UNEP, United Nations Environment Programme, 2009). As a result of their widespread occurrence in the environment and their reported possible adverse health effects, BFRs have become the subject of intensive research (Covaci et al., 2006).

Concentrations of PCBs and DDTs have generally been decreasing in the environment and in humans during the past few decades due to the decline of their use and production (Jones and de Voogt, 1999). PCBs have been recognised as endocrine-disrupting chemicals (Soontornchat et al., 1994; Maria et al., 2006; Geeraerts and Belpaire, 2010), and have more specifically been shown to directly impair eel larvae development (Palstra et al., 2005).

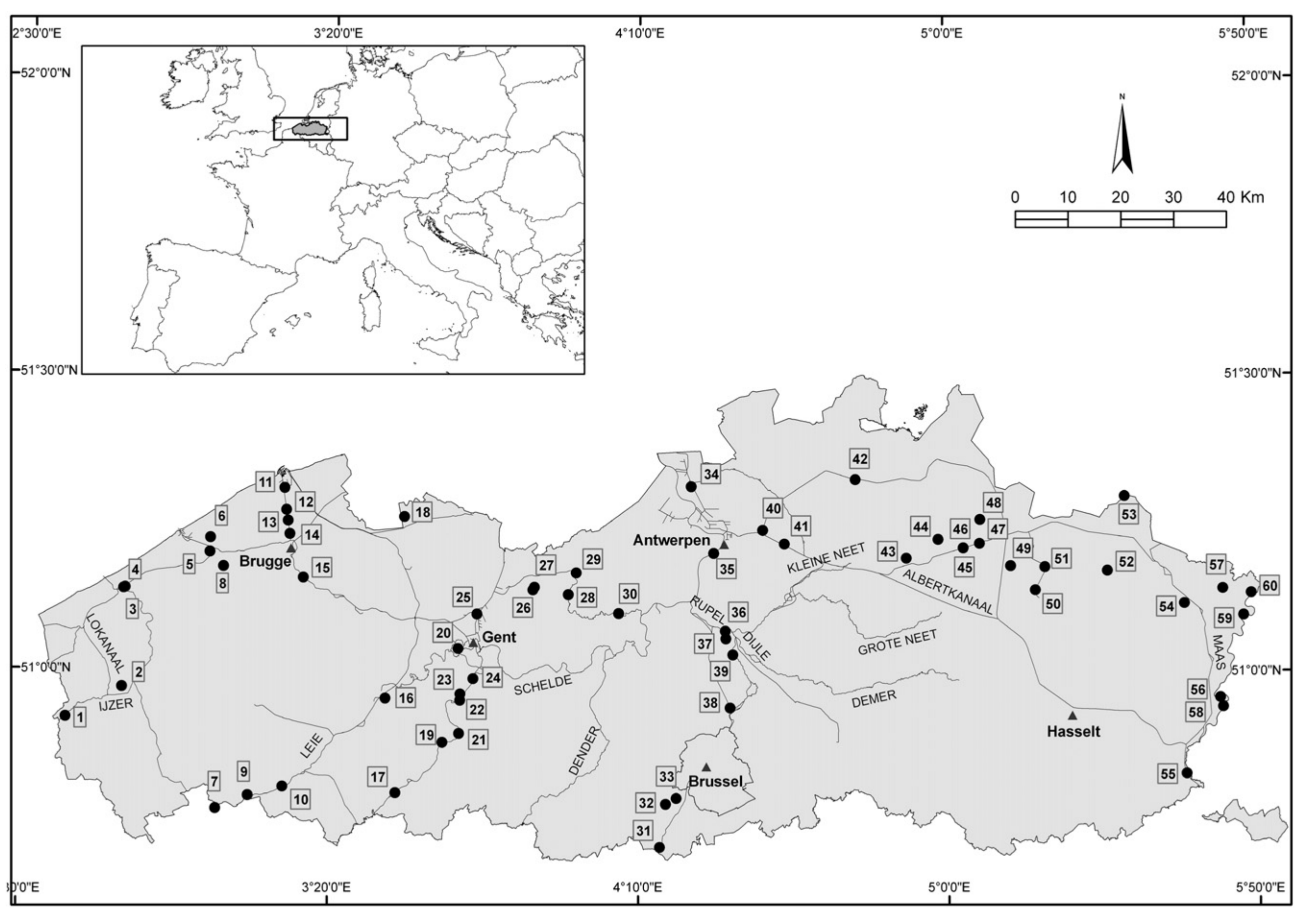

Fig. 1. Map of Flanders showing the sampling locations. Pooled yellow eel samples, consisting of 3–10 eels, collected between 2000 and 2009 from 60 locations in Flanders (Belgium). Numbers refer to description in Table 1.

Table 1

Concentrations of PBDEs, HBCDs, PCBs and DDTs in pooled eel samples from the present study. This study was carried out in Flanders (Belgium), where yellow eels (n = 443) were collected in 60 locations distributed over the catchments of the rivers IJzer, Scheldt and Meuse and in canals around Bruges and Ghent in the period 2000–2009. One pool containing between 3 and 10 individual eels has been prepared per location. An amount ranging from 1 to 4 g of muscle tissue (mid part of the body) was pooled.

No.	Sampling site	Water body	Water type	Lambert X coordinate	Lambert Y coordinate	Sampling year	*n*	Length (cm)		Weight (g)		Lipid (%)	PBDEs	HBCDs	PCBs	DDTs	ICES 6 PCBs	ICES 6 PCBs
								Mean	Range	Mean	Range		ng/g (lw)				ng/g (lw)	ng/g (ww)
1	Hoogstade	Ijzer	River	27,264	180,335	2005	8	34	30–39	73	50–117	10	46	28	840	405	276	28
2	Lo	Grote Beverdijk	Canal	37,963	185,902	2000	3	39	36–44	108	79–152	14	55	7.0	213	181	94	13
3	Nieuwpoort	Kanaal Nieuwpoort-Plassendale	Canal	38,365	204,400	2000	5	39	38–41	111	103–122	13	46	29	1323	323	577	73
4	Nieuwpoort	Kreek van Nieuwendamme	Creek	38,691	204,509	2002	7	35	32–43	77	58–132	11	14	15	233	230	102	11
5	Bredene	Kanaal Nieuwpoort-Plassendale	Canal	54,734	211,100	2001	9	37	30–41	98	48–139	22	52	237	1428	222	551	123
6	Bredene	Noord-Ede	Canal	54,849	213,770	2000	5	39	38–41	111	103–122	12	12	44	365	130	170	20
7	Wervik	Leie	River	55,611	163,050	2003	3	51	34–60	250	63–407	9.4	165	1070	8441	579	3478	329
8	Bredene	Hoge dijken (Roksem put)	Lake	57,295	208,390	2003	8	40	32–50	103	39–173	3.7	60	37	1173	530	491	18
9	Menen	Oude Leiearm	Old river arm	61,770	165,480	2004	10	50	38–55	233	103–293	9.3	135	53	5348	189	1950	181
10	Mouscron	Oude Leie Leiebos	Old river arm	68,320	167,090	2000	5	46	40–54	182	103–267	12	102	318	1372	229	493	57
11	Heist	Boudewijnkanaal	Canal	68,935	223,028	2006	6	40	26–54	139	26–312	7.1	16	212	2172	112	964	69
12	Heist	Boudewijnkanaal	Canal	69,251	218,910	2006	10	42	31–49	147	47–240	5.3	51	269	1895	123	831	44
13	Brugge	Boudewijnkanaal	Canal	69,539	216,874	2000	5	42	38–48	122	105–174	6.8	83	1271	4695	289	2022	137
14	Brugge	Boudewijnkanaal	Canal	69,862	214,377	2000	5	40	36–43	103	78–123	16	129	2979	1886	277	811	126
15	Loppem	Kanaal Gent-Oostende	Canal	72,396	206,215	2004	10	42	36–52	126	75–245	11	128	9494	3999	546	1444	159
16	Dentergem	Oude Leie	Old river arm	87,875	183,560	2000	5	38	36–41	97	70–137	18	75	78	3493	1122	1547	279
17	Avelgem	Oude Schelde	Old river arm	89,743	165,845	2002	5	40	33–46	112	65–151	3.4	62	81	757	1865	371	12
18	Eeklo	Hollandersgatkreek	Lake	91,582	217,566	2003	5	38	33–43	105	87–133	6.5	46	30	188	294	78	5.0
19	Horebeke	Oude Schelde Den Heuvel	Old river arm	98,660	175,260	2000	10	42	35–49	124	70–193	5.1	20	31	1064	2029	485	25
20	Gent	Blaarmeersen	Lake	101,716	192,850	2004	10	42	38–47	124	84–190	7.6	117	301	2683	241	1159	88
21	Horebeke	Oude Schelde Mesureput	Old river arm	101,826	176,940	2000	10	41	35–49	121	64–186	13	218	351	415	203	187	24
22	Gavere	Oude Schelde Teirlinckput	Old river arm	102,040	183,150	2000	10	37	22–50	103	66–213	7.2	57	77	1138	1514	514	37
23	Gavere	Oude Schelde Doornhammetje	Old river arm	102,085	184,325	2000	4	38	37–40	97	87–104	9.4	19	42	669	7029	318	30
24	Gavere	Oude Schelde Zonneput	Old river arm	104,506	187,187	2001	7	36	32–44	81	43–138	7.5	554	290	2291	485	978	73
25	Evergem	Kanaal Gent-Terneuzen	Canal	105,301	199,304	2006	8	41	35–47	132	72–187	9.9	224	7544	10,949	534	4155	413
26	Zeveneken	Bosdamvijver	Lake	115,900	203,785	2000	9	41	35–48	134	76–211	6.2	18	33	266	406	124	7,6
27	Zeveneken	Klaverbladvijver	Lake	116,200	204,330	2000	7	46	29–60	205	31–414	17	41	139	6197	610	2648	461
28	Lokeren	Moervaart	Canal	122,603	202,930	2006	10	41	35–44	115	76–148	9.4	197	231	1595	590	675	63
29	Lokeren	Kanaal van Stekene naar Eksaarde	Canal	124,091	206,985	2006	9	45	36–49	156	69–203	9.8	416	481	2826	508	1160	114
30	Sint-Niklaas	Oude Durme	Old river arm	132,150	199,346	2002	8	40	36–48	115	70–204	11	28	27	1423	2323	667	73
31	Ittre	Willebroekse vaart	Canal	139,860	155,573	2007	7	44	34–49	148	55–214	8.6	64	101	11,585	238	4812	416
32	Halle	Groot Zuunbekken	Lake	141,010	163,660	2002	10	40	34–43	97	62–131	3.8	34	38	1614	457	724	28

33	Halle	klein Zuunbekken	Lake	143,010	164,740	2002	10	40	35–44	107	78–145	15	21	31	4541	259	2027	302
34	Doel	Antwerpse dokken	Docks	145,866	223,129	2000	5	56	52–60	348	263–465	12	147	181	5330	317	2552	297
35	Antwerpen	Beneden Zeeschelde	Estuary	150,152	210,616	2000	5	38	30–53	102	42–250	6.7	1398	1159	9929	697	4273	286
36	Boom	Rupel	River	152,324	196,076	2007	10	38	31–46	110	50–181	12	138	304	7845	292	3146	372
37	Boom	Hazewinkel, roeivijver	Lake	152,440	194,640	2000	7	36	32–49	71	46–148	22	39	45	513	196	229	51
38	Vilvoorde	Willebroekse vaart	Canal	153,256	181,684	2002	10	40	36–45	103	67–149	10	110	151	9183	460	3454	361
39	Leest	Zenne	River	153,753	191,636	2008	10	40	35–47	117	76–198	8.9	126	273	10,282	260	4306	381
40	Borgerhout	Kanaal van Dessel naar Schoten	Canal	159,400	214,956	2009	9	70	56–84	576	253–1008	19	61	189	16,995	454	7933	1505
41	Schilde	Groot Schijn	River	163,518	212,392	2002	8	34	32–38	87	50–135	13	70	62	598	349	219	29
42	Oostmalle	Kanaal van Dessel naar Schoten	Canal	176,954	224,425	2003	8	48	36–60	210	81–374	9.2	71	155	26,322	374	12,499	1154
43	Kasterlee	Kleine Neet	River	186,620	209,751	2000	5	38	30–50	108	37–224	8.7	51	49	466	405	196	17
44	Kasterlee	Kleine Nete	River	192,625	213,274	2003	10	44	35–50	142	37–283	12	51	43	529	307	228	27
45	Retie	Kanaal Bocholt-Herentals	Canal	197,408	211,673	2002	10	41	36–45	115	79–152	7.0	51	112	40,378	503	21,128	1484
46	Retie	Kanaal Bocholt-Herentals	Canal	197,408	211,673	2007	6	44	40–48	140	104–180	13	36	30	30,937	318	16,036	2072
47	Retie	Congovaart + lagune	Canal/lake	200,465	212,535	2001	9	43	33–56	163	64–333	13	69	163	41,567	248	20,671	2595
48	Retie	Witte Nete	River	200,534	217,006	2007	6	49	39–55	206	109–294	9.6	13	25	248	418	105	10
49	Mol	Mol Neet	River	206,375	208,350	2007	7	42	33–54	160	47–309	10	47	72	838	1131	365	36
50	Leopoldsburg	Kanaal van Beverlo	Canal	211,084	203,852	2006	10	41	36–45	110	72–161	5.3	20	28	1683	416	705	38
51	Leopoldsburg	Kanaal van Beverlo	Canal	212,872	208,162	2006	3	48	37–53	207	91–285	3.7	39	60	3631	433	1470	54
52	Peer	Dommel	River	224,724	207,519	2007	4	46	31–62	225	51–457	8.6	50	175	480	177	203	18
53	Beverbeek	Warmbeek	River	227,910	221,484	2000	5	40	37–45	92	76–115	4.5	65	57	3669	339	1685	76
54	Bree	Itterbeek	River	239,296	201,443	2005	8	39	32–45	116	61–187	6.8	42	119	321	477	146	10
55	Veldwezelt	Albertkanaal	Canal	239,823	169,559	2000	6	42	33–50	131	58–202	11	111	123	18,184	358	7171	766
56	Rekem	Grensmaas	River	246,151	183,846	2005	10	40	35–46	113	64–162	11	87	124	14,466	250	5057	563
57	Maaseik	Itterbeek	River	246,580	204,271	2000	7	53	35–68	296	63–654	16	68	37	1549	446	678	109
58	Rekem	Grensmaas	River	246,706	182,145	2002	6	53	39–60	291	105–456	13	109	78	25,436	260	7619	996
59	Ophoven	Grensmaas	River	250,514	199,306	2006	6	40	33–48	120	66–202	2.2	123	541	15,539	410	6680	147
60	Ophoven	Grindplassen	Lake	251,945	203,475	2006	10	34	32–38	87	50–135	7.0	68	94	6925	171	3044	214
Mean												10	110	510	6380	590	2800	290
Median												10	60	100	2050	370	890	75
Standard deviation												4.5	190	1570	9450	950	4550	510
Minimum												2.0	12	7.0	190	110	78	5.0
Maximum												22	1400	9500	41,570	7000	21,128	2600

ICES 6 PCBs: CB 28, 52, 101, 138, 153 and 180.
ww — wet weight.
lw — lipid weight.

In Flanders, explicit concern was raised in order to warn recreational fishermen for the health hazards associated with the consumption of eel and other predatory fish with elevated POP levels (Hoge Gezondheidsraad, 2005). Many of these chemicals are considered potential carcinogens and some are believed to disturb metabolic and endocrine functions of the human body (European Environment Agency, 2005). Since fish is an important part of the human diet, the possible impact on human exposure to contaminants through fish has to be closely monitored. Also eels are a favourite food item in the Flemish population, but POPs have been reported to accumulate to a significant extent in the fat tissue of this species (Hodson et al., 1994; Ashley et al., 2007; Belpaire et al., 2009; de Boer et al., 2010; McHugh et al., 2010; Szlinder-Richert et al., 2010; Roosens et al., 2010; Belpaire et al., 2011).

The yellow eel stage is often chosen as a bio-indicator for the monitoring of environmental contaminants for several reasons, one being that this stage is characterised by primarily sedentary behaviour (Belpaire and Goemans, 2007). Their pollution load is thus expected to be indicative of the contaminant loading of the site where they live (Van Ael et al., 2014). Within their on-growing habitat, movements of yellow eels (foraging behaviour or other) seem to be very limited, and they reflect the contamination present in this particular site (Belpaire and Goemans, 2007). Eel contaminant profiles, especially for lipophilic substances, appeared to be a fingerprint of the contamination pressure of a specific site (Belpaire and Goemans, 2007; Roosens et al., 2010; Belpaire et al., 2011).

The aim of the present study was to investigate the current levels, spatial distribution and profiles of PBDEs, HBCDs, PCBs and DDTs in wild yellow eels from the freshwater system of Flanders, Belgium. Additionally, a risk assessment for consumers of eels was carried out.

2. Material and methods

2.1. Sample collection

Flanders, the northern part of Belgium, constitutes an area of 13,522 km^2, with a dense population (474 inhabitants/km^2; Eurostat EC, 2014), intensive agricultural and industrial activities. Yellow eels ($n = 443$) were collected between 2000 and 2009, from 60 locations in Flanders (Belgium) by the Research Institute for Nature and Forest (INBO). Sampling locations were characterised as rivers or brooks, canals, polder water courses or closed water bodies such as old meanders, ponds or lakes (Fig. 1). Eel samples were collected using fyke nets or electro-fishing techniques. Between 3 and 10 eels were caught per location, skinned and filleted, and an amount ranging from 1 to 4 g of muscle tissue (mid part of the body) was pooled and stored at −25 °C until analysis. Detailed information on the number of eels per pooled sample, sampling year, the size range, weight range and lipid percentage are given in Table 1.

2.2. Chemical analysis

The method for the analysis of 14 PBDEs (IUPAC numbers: BDE-28, -49, -47, -66, -85, -99, -100, -153, -154, -183, -196, -197, -203 and -209), 3 HBCDs (α-, β- and γ- isomers), 39 PCBs (IUPAC numbers: CB-18, -28, -31, -44, -47, -49, -52, -66, -74, -87, -95, -99, -101, -105, -110, -118, -128, -132, -138, -146, -149, -151, -153, -156, −157, -170, -171, -172, -174, -177, -180, -183, -187, -194, -195, -199, 196/203, -206 and -209) and DDTs (5 compounds: *p,p′*-DDE, *o,p′*-DDD, *o,p′*-DDT, *p,p′*-DDD and *p,p′*-DDT) in eel was similar to that described previously by Roosens et al. (2010) and Belpaire et al. (2011). Briefly, a homogenised sample of approximately 1 g pooled eel muscle was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (CB 143, BDE 77, BDE 128, ^{13}C-BDE 209, and ^{13}C-HBCDs). Further, the samples were extracted for 2 h by hot Soxhlet with 100 mL hexane/acetone (3:1, v/v) and cleaned-up on acidified silica. The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on ~8 g acidified silica (44%) and eluted with 20 mL hexane and 15 mL dichloromethane. The cleaned extract was evaporated to dryness, re-dissolved in 0.5 mL hexane and eluted from pre-packed silica cartridges (Varian: 500 mg/3 mL). The first fraction (A) eluted with 6 mL hexane contained PBDEs, PCBs and DDTs, while the second fraction (B) eluted with 8 mL DCM contained HBCDs. Both the fractions were evaporated to incipient dryness and re-dissolved in 100 μL *iso*-octane (Fraction A) and 100 μL methanol (Fraction B), respectively. Quantification of PBDEs, PCBs and DDTs was done using GC-MS, while HBCD isomers were quantified by LC-MS/MS (Roosens et al., 2010; Belpaire et al., 2011). Abbreviations are expressed as follows: PBDEs as the sum of 14 congeners, HBCDs as the sum of 3 isomers, PCBs as the sum of 39 congeners, 6 PCBs as the sum of 6 indicator PCB congeners (CB 28, 52, 101, 138, 153 and 180) and DDTs as the sum of 5 compounds.

2.3. Quality assurance/quality control

The extraction, clean up, and fractionation steps were evaluated by measurement of the absolute recoveries of the internal standards. The peaks were quantified as target compounds if (1) the retention time matched that of the standard compound within ±0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. The limit of quantification (LOQ) was calculated as three times the standard deviation of the mean of the blank measurements. Procedural blanks were analysed simultaneously with every batch of seven samples to check for interferences or contamination from solvent and glassware. Procedural blanks were consistent (RSD < 30%) and therefore the mean value was calculated for each compound and subtracted from the values in the samples. Mean ± SD recoveries of the internal standards PCB 143 and BDE 77 were 86 ± 6% and 93 ± 10%, respectively. The analytical procedures were validated through the analysis of certified material SRM 1945 (organic contaminants in whale blubber) for which deviations from certified values were less than 10%.

2.4. Statistical analysis

Statistical treatment of the obtained results was performed with the SPSS software (SPSS for Windows: SPSS Inc., 2001). Outliers were identified using box plots and confirmed by Grubb's test. For statistical analysis, concentrations below the LOQ (very few in number) were assigned a value of 1/2 ∗ LOQ. The Spearman rank correlations were used to examine the strength of associations between parameters. The results are presented as mean and median with minimal and maximum values. Parameters with a probabilistic value of <0.05 were considered as having a significant relationship with contaminant level. The concentration of PBDEs, HBCDs, PCBs and DDTs is expressed in ng/g lipid weight (lw), unless otherwise specified.

All statistics were performed using R 3.0.1 (R Core Team, 2013) and XLStat 2013.4.08 (Addinsoft, Paris, France). Shapiro–Wilk's test for normality and visual inspection of QQ-plots indicated the necessity to transform pollutant data according to log_{10}. Linear mixed effect models (LMEM) included the variable 'sampling site' as a random factor. The R^2 for the LMEM was represented by the R^2 of the correlation between the observed values and the model's fitted values. For each LMEM, normality of the residuals and influential outliers were investigated as well. Principal component analysis (PCA) on the lipid content and pollutant concentrations was based upon the Pearson correlation matrix. As such, biplots depict Pearson correlations between the compounds and the principle components (PC).

PCA was performed using XLStat version 2012.6.02 (Addinsoft, Paris, France). All concentrations were transformed according to $\log_{10}(X + 1)$ to assure normality of the data. Eigenvalues of all principle components (PC) were larger than 1. Although more PCs showed eigenvalues larger than 1, for brevity, biplots were only based upon the first two PCs.

3. Results and discussion

3.1. Lipid contents

The lipid percentage varied widely and ranged between 2.0 and 22% ($n = 60$), with a mean and median value of 10%. After removal of years with only one observation (2008 and 2009), the lipid content decreased significantly over time ($t = -2.49$; $p = 0.04$; $R^2 = 0.10$; $n = 58$ (Fig. SI-1). Making such temporal comparison, possible confounding by different habitats and individual ages may however have occurred. A similar percentage (mean: 10% and median: 9.5%) in fat content of yellow eels collected between 2000 and 2007, from 48 locations in Flanders was observed recently (Belpaire et al., 2011). In Belgium, the relationship between lipid content and various environmental variables was studied by analysing extensive datasets of contaminants. It was demonstrated that PCBs (especially higher chlorinated ones) had a negative impact on the lipid content of the eel (Geeraerts et al., 2007). Although eels were sampled during their yellow stage, the lower fat contents may have their implications also for silver eels. Lipid reserves are essential to cover energetic requirements for silver eel migration and reproduction. It is hypothesised that after silvering, eels with insufficient fat content (<13% of the body weight) will be incapable to fulfil their reproductive migration, or will not even start their journey. A value of 20% (of the body weight) fat is also the average for migrating silver eels, implying that only half of the silver eels are capable of successful migration and reproduction (Burgerhout et al., 2013). Establishing sufficient lipid energy is thus essential in the life cycle of the eel. Analysis of fat content ideally should include the whole eel carcass in order to get a correct estimate of total fat stores. The eel body is known to vary with respect to fat content depending on where on the body the sample is taken, the tail being the fattest part (Tesch, 2003). However, care must be taken when comparing literature data on eel fat levels between studies, as methodological and analytical issues might vary to some extent and most often the detailed description is missing.

3.2. Contamination status of BFRs

The median value for total PBDEs for the 60 sites was 60 ng/g lw, ranging between 12 and 1400 ng/g lw (Table 1; Fig. SI-2). An even higher variability in contamination level was seen for HBCDs, ranging between 7 and 9500 ng/g lw, with a median value of 100 ng/g lw (Table 1; Fig. SI-3). This clearly shows that HBCD distribution patterns showed a more widespread usage compared to PBDEs. Possibly, this is associated with regulatory measures taken for both BFRs, and have been reflected in other biota such as in harbour porpoises too (Law et al., 2008).

The broad range of contaminants reported in this study is likely due to the diversity of the sampling locations, from highly industrialised areas to small rural creeks. Excepting the higher values, concentrations of PBDEs and HBCDs are in line with what has been observed in previous studies in Flanders, Belgium (Roosens et al., 2010). The lowest levels of PBDEs were recorded at sites located in rural areas with low industrial activities (Bredene-site 6, Heist-site 11, and Horebeke-site 19), with levels between 12 and 20 ng/g lw (Fig. SI-2). Eels collected from Antwerp region in 2000 contained the highest levels (a maximum PBDEs of 1400 ng/g lw). This is likely because this particular sample location was located in the estuary of the Scheldt River, the main river collecting effluents from various textile and industrial activities upstream the Scheldt water basin (Voorspoels et al., 2003, 2004). For HBCDs, the lowest levels are recorded at sites located in rural areas (Lo-site 2, Nieuwpoort-sites 3 & 4, and Eeklo-site 18), with levels between 7 and 30 ng/g lw (Fig. SI-3). Eels collected from Loppem (site 15) in 2004 and Evergem (site 25) in 2006 contained the highest levels (maximum HBCDs of 9500 and 7540 ng/g lw). Although samples with high PBDE content typically contained higher levels of HBCDs, the opposite was not always true, which indicates a different usage of both BFRs.

Comparisons between studies are likely to show large variations in BFR concentrations, as these depend largely on the sampling location and on the year of sampling, as levels tend to stabilise or decline due to regulatory measures (Law et al., 2008). Our data refers to a 'random' monitoring network, whereas in many other reports/studies the data refers to samples from particular sites of concern (or types of sites e.g. such as in the Netherlands, the large rivers). Bragigand et al. (2006) analysed PBDEs in eel originating from two rivers in France (Seine and Loire) with a concentration range between 26 and 108 ng/g lw, and a median value of 40 ng/g lw. Van Leeuwen and de Boer (2008) analysed eel samples to estimate their contribution to human PBDE intake. The levels of Σtri-hepta PBDE congeners ranged between 3 and 3139 ng/g lw, with a median value of 261 ng/g lw indicating the presence of some highly contaminated eel samples with concentrations between 1000 and 3000 ng/g lw, but the majority of the samples contained a median value of 200 ng/g lw, slightly higher than our results. No conclusive explanation was given for these higher concentrations although sampling of larger, more industrialised waters is thought to lead to more contaminated biota (Van Leeuwen and De Boer, 2008). PBDE levels in the eels from our study are high in comparison with data from Gironde estuary, France (Tapie et al., 2011), Elbe river, Germany, Lake Ontario, Canada and the Saint Lawrence river, Canada (Sühring et al., in press). The range of the Flemish HBCD concentrations is similar to that seen recently in the Netherlands (Van Leeuwen and de Boer, 2008), but the median value is significantly lower, indicating the presence of more highly contaminated sampling sites in the Netherlands than in Belgium.

Among PBDEs, BDE 47 was the dominant congener in most eel samples (Fig. 2; proportion in relation to the sum of 7 PBDE congeners) and accounted on average for 60% of Σtri-hepta PBDEs, followed by BDE 100 (20%) and BDE 99 (15%). Ashley et al. (2007) reported similar PBDE profiles in American eel (*A. rostrata*) and suggested that high BDE 47 and low BDE 99 levels were a possible outcome of metabolic pathways, present in eels. Such pathways have been described for carp (*Cyprinus carpio carpio*) (Stapleton et al., 2006). It has been shown that BDE-47 is the predominant congener that bioaccumulates in freshwater fish species (de Wit, 2002). A possible reason for this could be the higher uptake efficiency for BDE-47 from the environment (Burreau et al., 2000). The predominance of BDE-47 in the present study is consistent with the general pattern found in biota samples in other studies (Labandeira et al., 2007; Peng et al., 2007), and is mainly due to the fact that BDE-47 is one of the major components of penta-BDE formulation which was used in many countries. Relatively lower amounts of BDE-99 compared to BDE-100 were observed in this study which is in agreement with Hale et al. (2001) in freshwater fishes from Virginia, USA. Another plausible explanation is the biotransformation of BDE-99 to BDE-47 within fish tissue, a hypothesis proposed by Stapleton et al. (2004).

Haglund et al. (1997) reported that metabolic differences, age of the fish and lipid content as well as other physiological differences among fish species are possible reasons for observed PBDE patterns. PBDEs enter coastal waters through municipal and industrial wastewater outfalls, land fill leachate and atmospheric deposition from multiple sources (de Wit, 2002). However, land runoffs compared with atmospheric deposition may be considered as one of the most important pathways for PBDEs entering the water bodies. Due to the ban or restricted use of all the PBDE commercial mixtures in the EU, the recent exposure is most likely due to the release of PBDEs which have accumulated in environmental matrices, such as soils and sediments. Eels, primarily benthic feeders, are prone to accumulate pollutants from sediments in addition to other contaminant pathways. Thus the monitoring of sediment remains useful for further studies as indirect release of POPs in the environment is likely to become more important.

The fact that HBCDs are found in fish indicates its bioavailability and bioaccumulation potential. For HBCDs, α-HBCD was predominant (average 75%), followed by γ-HBCD (20%) and β-HBCD (4%) in almost

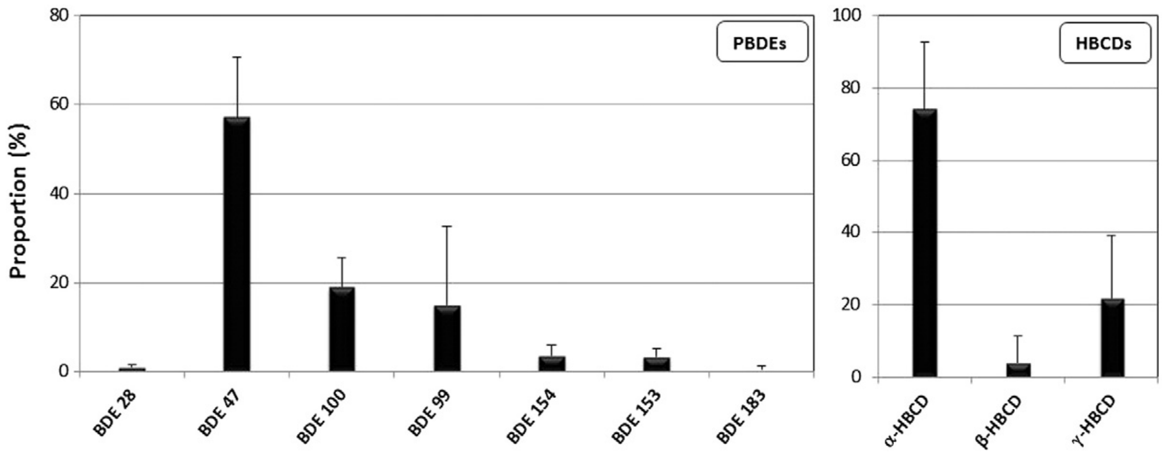

Fig. 2. Congener profile of PBDEs and isomer pattern of HBCDs in eel samples collected between 2000 and 2009 from 60 locations in Flanders (Belgium). PBDEs: proportion in relation to the sum of 7 PBDE congeners. HBCDs: proportion in relation to the sum of 3 isomers.

all the eel samples (Fig. 2; proportion in relation to the sum of 3 isomers). The predominance of α-HBCD in fish of the present study is congruent with the scientific literature (Xia et al., 2011; Covaci et al., 2006), indicating its higher bioaccumulative potential. Although the HBCD technical mixture is mainly composed of γ-HBCD, several factors have been suggested to explain the isomeric shift to α-HBCD, which is the most common isomer in biota. The following three factors might be responsible for this observation: 1) bio-isomerization of γ-HBCD to α-HBCD as observed in mice (Szabo et al., 2010), 2) α-HBCD has a higher water solubility (49 μg/L) than β- and γ-HBCD (2 μg/L) and thus, is more readily available for uptake (Hunziker et al., 2004) and 3) in vitro experiments with rat and harbour seal microsomes showed that the biotransformation of β- and γ-HBCD was faster than that of α-HBCD (Zegers et al., 2005). It is also possible that physico-chemical differences among the HBCD isomers contribute to this pattern: α-HBCD has a relatively higher aqueous solubility than γ-HBCD, which may result in some preferential uptake of α-HBCD in the aquatic environment via transfer from particles through the water phase into organisms (Morris et al., 2004).

PCA on organobrominated compounds, i.e. PBDEs and HBCDs, resulted in a first (43.16%) and second PC (14.69%), together explaining 57.85% of the observed variation in those compounds (Fig. 3). PC 1 shows a concentration gradient, indicating that most locations exhibit an unclear accumulation pattern, possibly reflecting background contamination. However, some locations attribute to elevated BFR concentrations, amongst which three groups can be distinguished. The first group shows locations, amongst which, sites with elevated higher brominated hepta- and octa-BDE congeners, the second group shows locations, amongst which, sites with elevated levels of tri- to hexa-BDEs, and the third group shows locations with elevated HBCD accumulation.

3.3. Contamination status of PCBs and DDTs

PCBs were the major contaminants in most of the samples. Levels of PCBs, which have longer history and larger amount of usage than DDTs and BFRs, were the highest followed by DDTs, PBDEs and HBCDs (Table 1). This might imply a higher persistence of PCBs coupled with a higher degree of pollution from anthropogenic activities. There was very high variability in PCB levels, ranging between 190 and 41,570 ng/g lw, with a median value of 2050 ng/g lw (Table 1; Fig. SI-4). The median value for total DDTs for the 60 sites was 370 ng/g lw, ranging between 110 and 7000 ng/g lw (Table 1; Fig. SI-5).

PCB distribution was positively skewed and variance around the mean was high due to very high concentrations at specific sites. Levels of PCBs are significantly ($p < 0.05$) lower with what has been observed previously (median value for the 50 locations was 4000 ng/g lw, ranging between 250 and 64,850 ng/g lw) in Flanders (Belpaire et al., 2011). Making such comparison, possible confounding by different habitats and individual ages may however have occurred. Long-time monitoring studies on eels in the Netherlands (since 1977) and Belgium (since 1994) indicated an overall significant declining trend in PCB levels (de Boer et al., 2010; Maes et al., 2008). The current PCB monitoring studies still report elevated levels in several locations with a high variability between sampling locations and river basin districts (Fig. SI-4). The lowest levels are recorded at sites located in rural areas with low industrial activities (Eeklo-site 18, and Lo-site 2), with levels between 190 and 210 ng/g lw. Staggeringly eels collected from Retie region (sites 45–47) in 2001, 2002 and 2007 contained the highest levels (a maximum PCBs of 41,567, 40,378 and 30,937 ng/g lw, respectively). PCB contamination in Flanders increased along the west–east gradient which is in agreement with earlier studies (Belpaire et al., 2011). The origin of the PCB contamination on Canal Dessel–Schoten (site 42) is diverse. The canal is fed with water from the river Meuse, and is a transport route for industries located on its banks. It was reported that PCBs are one of the major contaminants present in the river Meuse and some canals in the north-eastern part of Flanders (Belpaire et al., 2011). Local and upstream sources linked to industrial activities seem to be the main pathways for the presence of PCBs in Flanders.

The mean PCB 153 concentration of 125 ± 240 ng/g wet weight (ww) (range 2.0–1200 ng/g ww) measured in the 60 pooled eel samples was significantly higher compared to the reported mean levels from Italy, with mean PCB 153 of 42.8 ± 40.9 ng/g ww (range nd–143.21 ng/g ww) in eels from the Garigliano River (Ferrante et al., 2010) and 18.6 ± 2.9 ng/g ww (range 14.0–21.0 ng/g ww) in eels from the Lesina lagoon, Adriatic Sea (Storelli et al., 2007). In Spain, Turia river eels contain low mean PCB 153 body burdens of 5.9 ng/g ww (Bordajandi et al., 2003). PCB levels in the eels from our study are fairly high in comparison with data from Gironde estuary, France (Tapie et al., 2011), other French areas (Oliveira Ribeiro et al., 2008) or from Ireland (Santillo et al., 2005). However, variables, such as sampling strategy, number of sampling locations, sampled area and year should be kept in mind when comparing country specific contamination data, as they can influence the outcome significantly. This stresses the need for a comprehensive standardised study on the occurrence of POPs in European eels.

In all samples, CB 153 (19%) was the most dominant PCB congener, closely followed by CB 138 (11%), CB 180 (9%), CB 187 (8%) and CB 149 (7%), respectively (Fig. 4; proportion in relation to the sum of 30 PCB congeners). In Europe, PCBs 153 and 138 are the most dominant PCB congeners in eels, but the relative abundance of individual congeners in the samples varies depending on the origin and country considered. Flemish eels are characterised by a higher proportion of PCB 153

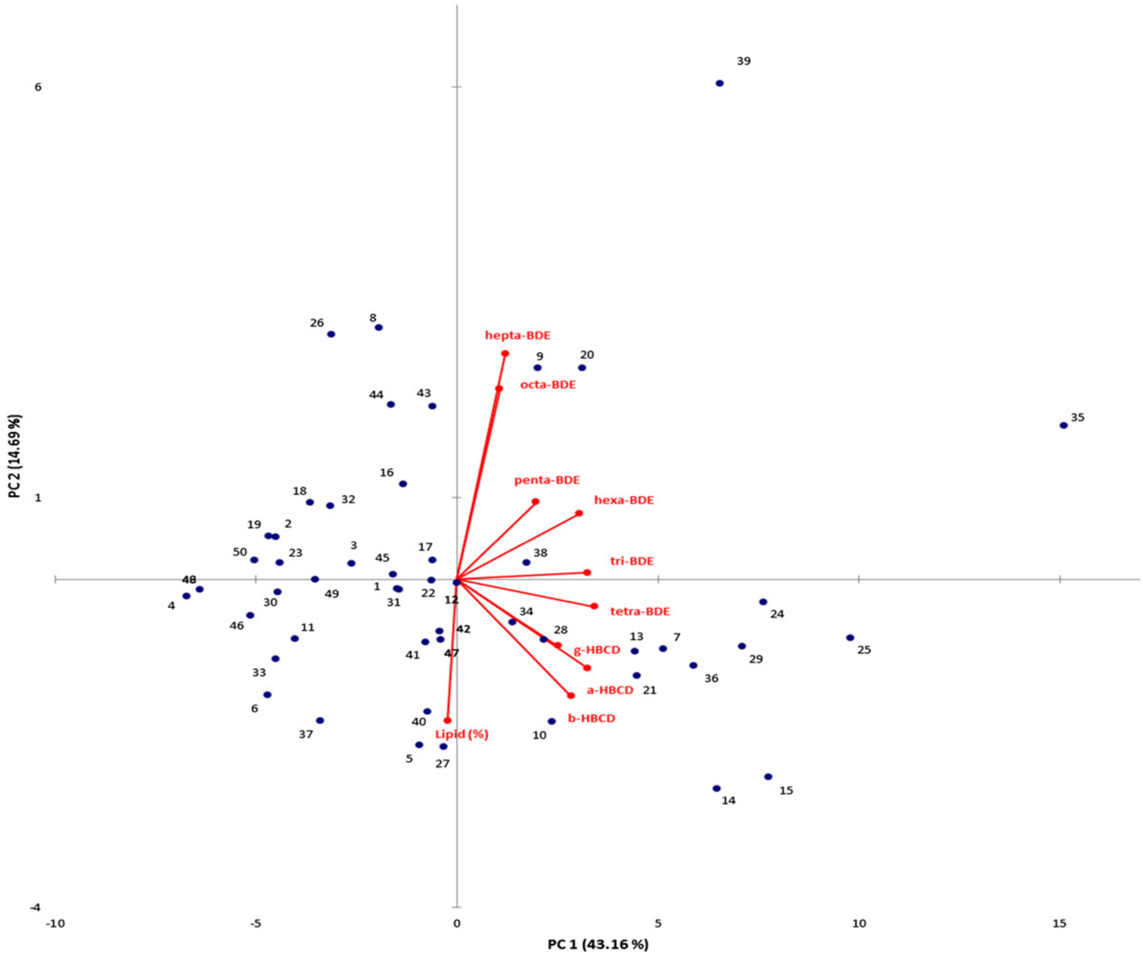

Fig. 3. Pearson correlation biplot of the PCA on brominated flame retardant concentrations in European eel *Anguilla anguilla* from Belgium ($n = 60$). Numbers refer to description in Table 1.

and PCB 180 as compared to other European countries, which was also described in former Belgian studies (Belpaire et al., 2011; Maes et al., 2008). The relatively high contribution of the less lipophilic lower chlorinated congeners in our samples (Fig. 4) is in line with previous studies on freshwater fish species from Spain (Bordajandi et al., 2003) and fishes from Shadegan wetlands, Iran (Davodi et al., 2011), which indicated the accumulation of lower congeners at significant proportions in air due to their high vapour pressure. The difference could also result from differences in the complexity and species included in the food web. Within Flanders, PCB composition also varied between sites. PCB composition indicates that a mixture of Aroclor 1260 and 1254 could be the source of pollution. The spatial variation in PCB composition in Flemish eels may be attributed to the local use of products with slightly different mixtures.

DDTs (median: 370 ng/g lw; range 110–7000 ng/g lw) were present in all the eel samples and the distributions of DDTs levels were also positively skewed. DDT levels were significantly lower ($p < 0.05$) with what has been observed previously in Flanders (Maes et al., 2008). The current DDT monitoring studies still report elevated levels in some locations with a high variability between sampling locations and river

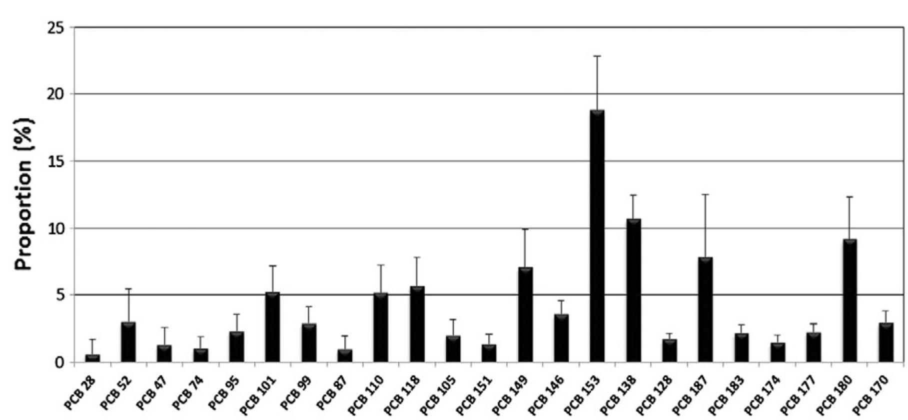

Fig. 4. Congener profile of PCBs in eel samples collected between 2000 and 2009 from 60 locations in Flanders (Belgium). PCBs: proportion in relation to the sum of 30 PCB congeners.

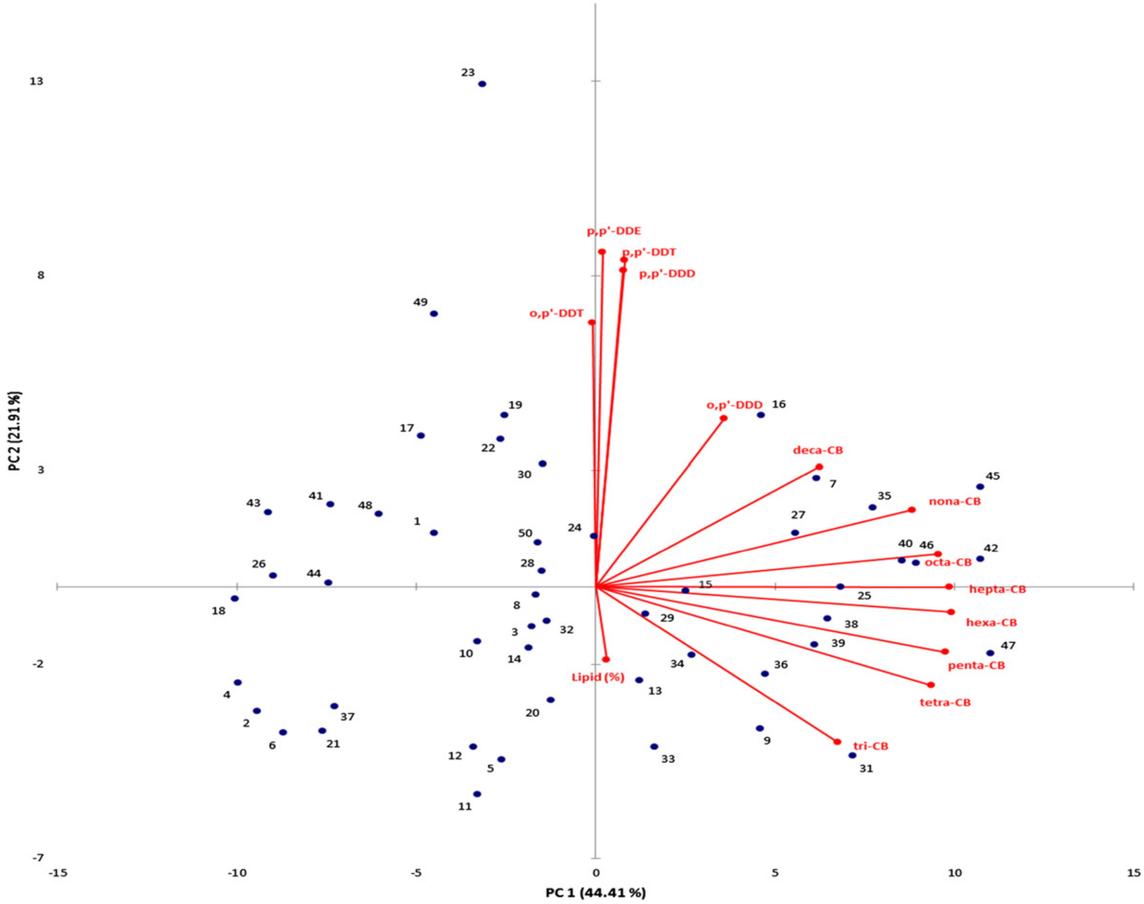

Fig. 5. Pearson correlation biplot of the PCA on organochlorine compound concentrations in European eel *Anguilla anguilla* from Belgium ($n = 60$). Numbers refer to description in Table 1.

basin districts (Fig. SI-5) and variability in derivate profiles, probably indicating recent application of DDT and showing that not all stock was depleted. Human milk (Colles et al., 2008) and adipose tissue (Covaci et al., 2002; Malarvannan et al., 2013), particularly of the human populations living outside urban areas, still contain DDT. This should urge regional policy makers to continue their efforts in collecting remaining stocks of banned pesticides.

Among DDT metabolites, *p,p′*-DDE was the predominant compound, followed by *p,p′*-DDD and *p,p′*-DDT, suggesting a wide usage in the past and long-term accumulation of DDTs in eel samples in all the locations in Flanders (data not shown). It is generally accepted that increased percentage of DDT metabolites (i.e., DDE and/or DDD) in fish samples reflected a decrease input of fresh DDT. The ratio of *p,p′*-DDE/*p,p′*-DDT suggested that the main source of DDTs was possibly due to the past agricultural and public health usage. Due to various biological processes, metabolism of DDTs may occur throughout the food chain and therefore the dominance of *p,p′*-DDE in eel samples is not only derived from direct ingestion of *p,p′*-DDT, but also from the ingestion of *p,p′*-DDE, previously degraded in the environment.

PCA on organochlorinated compounds, i.e. PCBs and DDTs, resulted in a first (44.41%) and second PC (21.91%), together explaining 66.31% of the observed variation in those compounds (Fig. 5). PC 1 shows a concentration gradient, which shows that again a large part of the locations does not show a specific accumulation pattern that can be related to a certain group of chlorinated compounds, thus possibly reflecting background contamination. One group of locations, amongst which, sites with high levels of *p,p′*-DDT and most metabolites, except for *o,p′*-DDD is separated from sites characterised by the different classes of PCB congeners, which are all highly inter-correlated.

3.4. Risk assessment for humans

Since fish is an important part of the human diet, the consumption of contaminated fish can lead to an unwanted increase in the body burden of contaminants. Eel consumption associated health risks may be a serious challenge for communities that largely depend on fish from contaminated water bodies. Dietary intake is an important pathway for human exposure to BFRs and fish contributes much to the total human dietary exposure (Voorspoels et al., 2007; Roosens et al., 2010). The consumption of fatty and polluted fish species, such as the eels discussed in the present study, can influence dietary exposure to a large extent. As there are no professional eel fisheries in Flanders, consumption of wild eel occurs mainly by recreational fishermen and their families. We calculated the daily intake of PBDEs, HBCDs, PCBs and DDTs via eel consumption for the average population (consumption of 2.87 g eel/day), as well as for fishermen who take their catch home for own consumption (71.14 g eel/day; Bilau et al., 2007). Any other scenario would lead to intakes ranging between the intakes calculated for the two above scenarios.

Table 2 shows dietary intakes of average population and the risk group normalised per body weight (an average of 70 kg was used for the calculations). These dietary intakes are compared to the tolerable daily intakes (TDI) or reference doses (RfD) for the various groups of contaminants. The TDI and RfD values have been calculated based on animal in vivo studies (Table SI-1). These are an estimate of daily exposures of the human population which are likely to be without an appreciable risk of deleterious effects during lifetime. Daily intake above 100 ng/kg/day BDE 47, 2000 ng/kg/day HBCDs, 20 ng/kg/day PCBs and 20,000 ng/kg/day DDTs is expected to cause health risks.

Table 2
Calculated human intake (ng/kg/day) of PBDE47, HBCDs, PCBs and DDTs through eel consumption for both the average Belgian population and the risk group of fishermen fishing at the studied sites. Eel consumption (g/day) was taken from Bilau et al. (2007).

	RfD (ng/kg/day)		Normal	Risk group
Eel consumption (g/day)			2.87	71
BDE47	100	Mean	0.25	6.2
		Median	0.13	3.3
		Range	0.01–2.58	0.33–63.9
HBCDs	2000	Mean	2.2	54.2
		Median	0.38	9.5
		Range	0.04–42.8	1.0–1060
PCBs	20	Mean	12.0	296
		Median	3.1	75.7
		Range	0.2–106	5.1–2637
DDTs	20,000	Mean	2.3	56.5
		Median	1.5	36.2
		Range	0.3–27.1	6.6–673

The average population (consumption of 2.87 g eel/day).
Risk group — fishermen always taking their catch to home for their own consumption (71.14 g eel/day).

The contribution of PCBs to the total human exposure through the consumption of local wild eel was highly variable across the different sampling locations. At 27% (16 on 60) of the sites, eels exceeded largely the new EU consumption threshold for 6 PCBs which is 300 ng/g ww for the sum of the 6 indicator PCBs: CB 28, 52, 101, 138, 153 and 180 (European Commission, 2011); data shown in Table 1 and Fig. 6). Also 14% of the sites (8 out of 60) were exceeding the TDI of PCBs (20 ng/kg body weight per day) for the average population consumption. For the high risk group of fishermen, 83% of the sites (50 out of 60) exceeded the TDI of PCBs.

Former studies concluded that the intake of PCBs via the consumption of self-caught eel is a cause for serious concern, and they recommended that stakeholders should discourage recreational fishermen from consuming their wild-caught eels by all legal and practical means (Belpaire et al., 2011). The mean sum of 6 PCBs measured in wild eels is 100 fold higher than the mean value of 7.1 ng/g ww in fish and seafood from the Belgian market (Voorspoels et al., 2008). PCB intake seems to be at a level of high concern, and body burden in fishermen in Flanders might reach levels of toxicological relevance (Roosens et al., 2008; Belpaire et al., 2011). Our data shows that local eel anglers consuming their own catch, especially from polluted sites, are at high risk.

4. Conclusions

Distribution of PCBs, PBDEs or HBCDs in Flemish eels varies widely between sampling sites and river basin districts, and alarming concentrations were found at industrialised hot spots. Intake of PBDEs and HBCDs, through consumption of contaminated eel, was below the RfD values for the average population, although the exposure of risk groups (e.g. recreational fishermen) may significantly exceeds these levels depending on the sampling locations. Eels from 16 out of 60 sites exceeded largely the new EU consumption threshold for the sum of the 6 indicator PCBs. The current data show an on-going exposure of Flemish eels to these lipophilic chemicals through indirect release from sediments or direct releases from various industries. Our results emphasise the previous advices to not consume eels, especially from the areas of concern. As a result, there is an urgent need to identify sources of exposure to these chemicals in Flanders, as well as to quantify emission and document their potential environmental fate in this region. With regard to the effect on the eel stock, significant gaps in scientific knowledge have

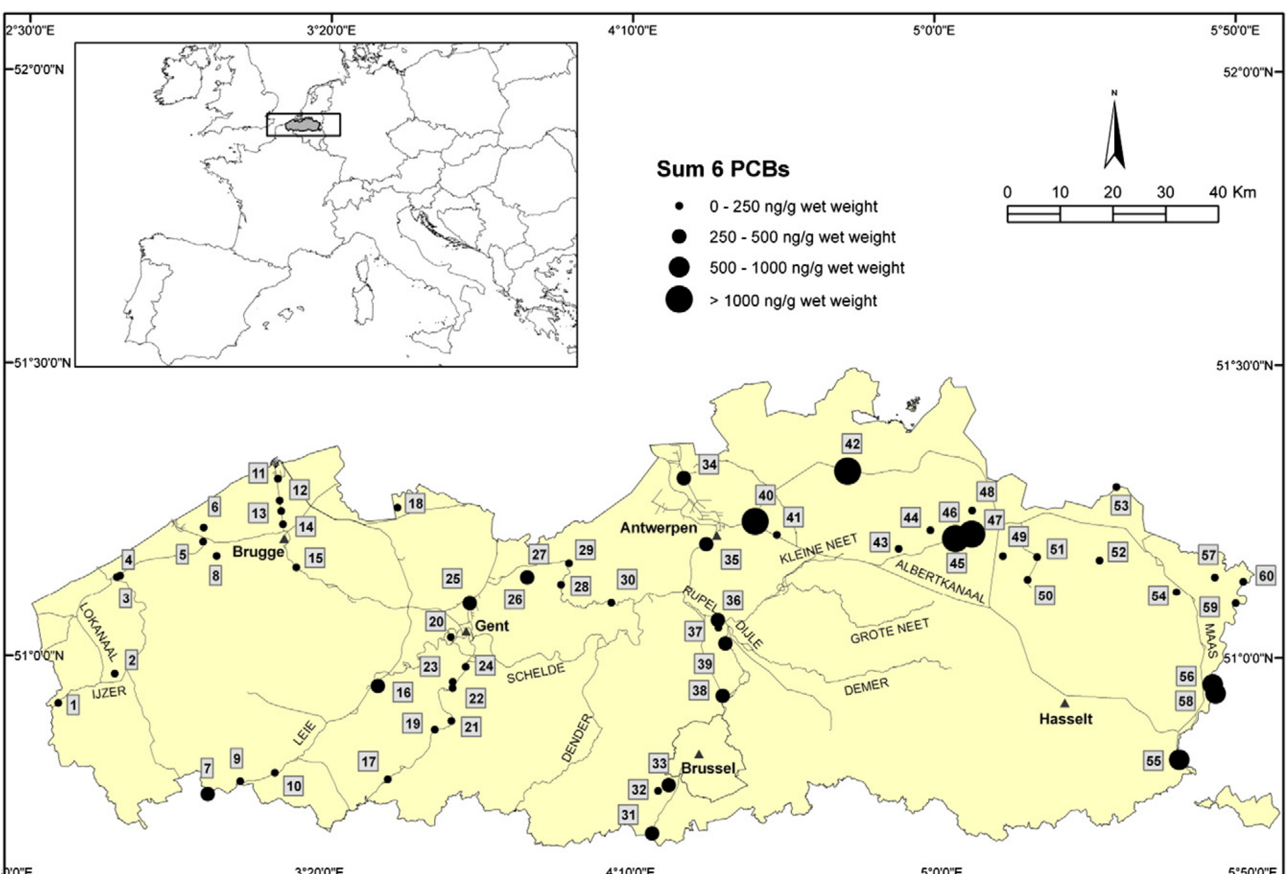

Fig. 6. Concentrations (ng/g ww) of ICES 6 PCBs in eels from 60 locations in Flanders, Belgium.

been recognised by ICES (2013), such as to what extent and at what level these contaminants affect the eel reproductive success.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organisations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.02.127.

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